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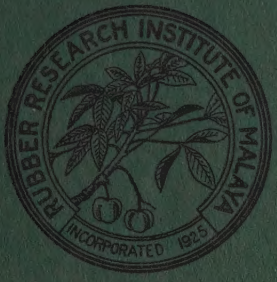

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A HITHERTO UNDESCRIBED LEAF DISEASE OF HEVEA RUBBER CAUSED BY A SPECIES OF COLLETOTRICHUM

by

K. P. John

Introduction

The earliest record of a *Colletotrichum* on *Hevea brasiliensis* is that of Petch (1906), who described a new species, *C. heveae*, as occurring on leaves of seedling *Hevea brasiliensis* in Ceylon. No record has been found in the literature of a *Colletotrichum* attacking *Hevea* leaves in Malaya until 1937, when Beeley reported a species of *Colletotrichum* as giving trouble in nurseries, and on young rubber in the field, in association with *Helminthosporium* and *Gloeosporium*.

Altson (1950*a*) reported that in 1947 a species of *Colletotrichum*, differing from Petch's *C. heveae*, had been occasionally found on seedling leaves suffering from a heavy attack of *Helminthosporium*. He also mentioned (1950*b*) the occurrence in 1948 of an anthracnose of rubber caused by *Colletotrichum ficus* Koorders.

Since then a species of *Colletotrichum* quite distinct from *C. ficus* Koord. and *C. heveae* Petch has been found frequently on mature leaves of young rubber. The fungus was also found occasionally to cause leaf spots on mature trees (8-year-old Buddings of Pilmoor B.84).

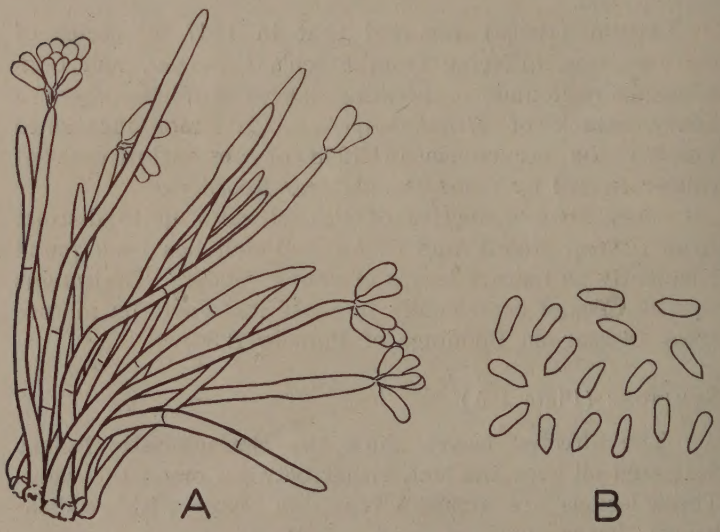
Symptoms (Plate I A)

The affected leaves show the characteristic lesions scattered all over the leaf, either between or on the veins. These lesions are almost circular in shape with reddish-brown lower surfaces and ash-brown upper surfaces. Their general appearance is grey or black due to the presence of numerous tufts of black setae arranged usually in concentric circles, especially towards the margin of the spot. The setae usually bear a few white glistening spores at their ends. The lesions may reach as much as 5.0 mm in diameter.

The fungus may account for all the spots of a leaf or may be associated with *Helminthosporium heveae.*

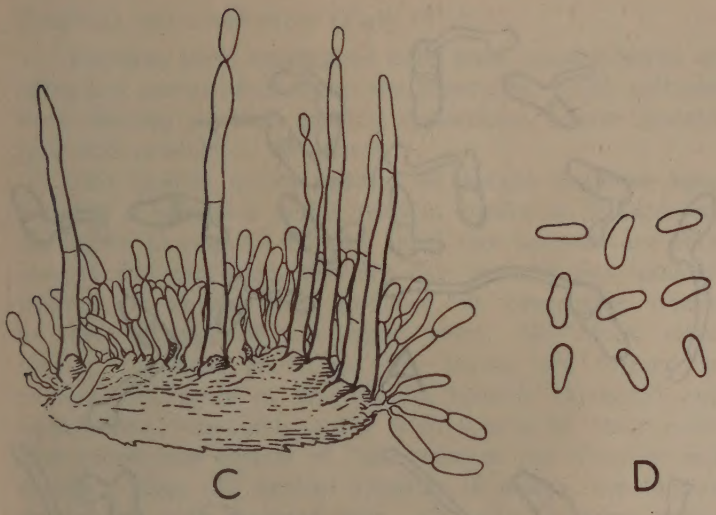
THE SETAE AND SPORES (Plate IV B and Text-fig. 1)

The setae are seen as black dots in the lesions and usually arise from acervuli, though occasionally one may be found unassociated with an acervulus. They are commonly in clusters, though sometimes solitary; up to 60 have been found in a cluster. They arise from special large, thick-walled, dark cells at the surface of the stroma and are dark brown at the base and almost colourless towards the tip. They are long, with usually 2—5 septa, and are bluntly pointed at their ends. About a quarter of their length from the tip there is usually a small bulge and sometimes a bend, this is particularly noticeable in specimens kept overnight in a damp chamber. The length varies from 84—170 μ (with an average length of 133 μ) and the width from 4—5 μ. In the natural state conidia are borne in clusters at their tips.



Text-fig. 1.

A. A tuft of setae showing conidia; B. Spores from setae;



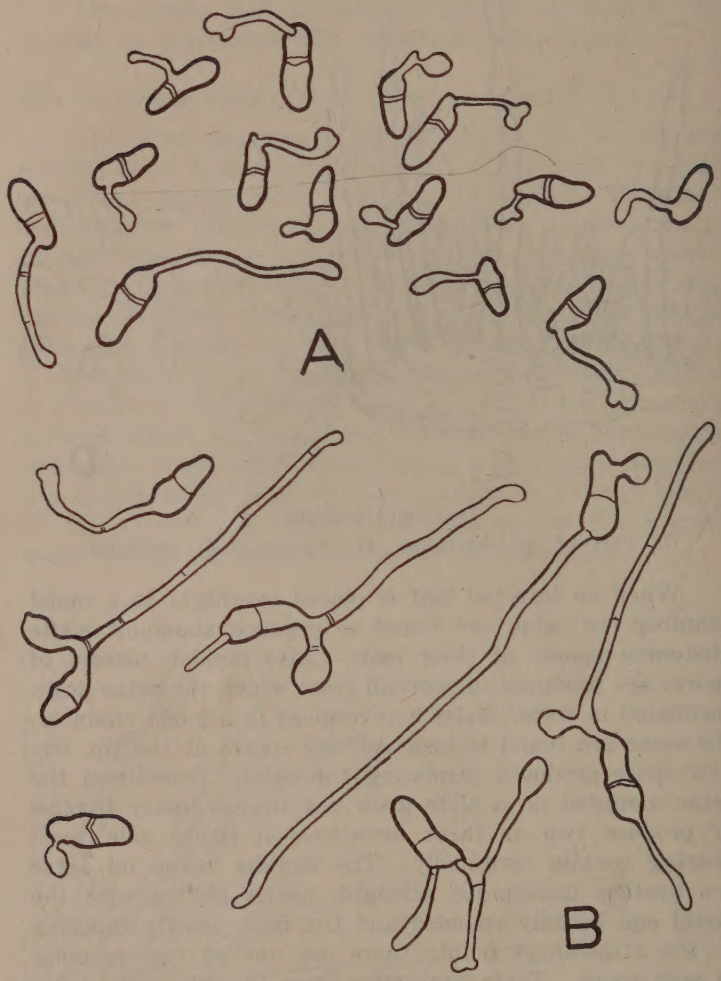
Text-fig. 1 (contd.).

C. Part of an acervulus; D. Spores from acervulus.

When an infected leaf is placed overnight in a moist chamber the setae are found to produce abundant white glistening spores at their ends. Also pinkish masses of spores are produced in acervuli from which the setae arise. Incubated in water under a coverglass in a moist chamber, the setae are found to bear solitary spores at the tip, any new spore produced displacing the older. Sometimes the setae mounted on a slide grow out into ordinary hyphae or produce two or three branches on either side, each bearing conidia terminally. The conidia borne on setae are hyaline, nonseptate, straight, nearly oblong with the distal end broadly rounded and the base usually tapering to the attachment point; there are one or two vacuoles in each spore. Their size varies from 13—20 μ x 4.5—8 μ with an average of 16.1 x 6.6 μ (for 20).

GERMINATION OF SPORES (Text-fig. 2)

The spores are found to germinate about an hour after mounting in distilled water on a slide. In five hours some of the germ tubes are as much as four times the length of a conidium. At the time of germination most of the conidia become one-septate.



Text-fig. 2.

A. Germinating spores after 5 hours, showing germ tubes and appressoria; B. Germinating spores after 24 hours.

In the majority of cases the distal cell produces the first germ tube. One of the germ tubes, usually the first, invariably rounds off to form the appressorium; the other usually develops into an ordinary slender hypha, 3—4 μ broad. Sometimes the appressorium itself develops into an ordinary hypha, or into a brown-coloured chlamydospore. In 24 hours some of the germ tubes grow as long as 0.8 mm. The usual number of germ tubes produced by a spore is two; three are fairly frequent, but one is rare.

CULTURAL CHARACTERISTICS (Plate IV A)

Cultures were established both from conidia borne on setae and spores produced in the acervulus. Both cultures were identical in their general appearance, colour, growth rate and production of spores.

The fungus grows rapidly on potato dextrose agar forming a luxuriant aerial growth, woolly in texture and dark-grey in colour. By the third day acervuli are seen around the inoculum, and by the seventh day pinkish masses of spores are produced in concentric circles on the surface of the agar slant. No black setae similar to those found on the leaves are formed in culture, but some of the basidia become elongated and light-brown resembling setae with spores at their ends. The spores are typical of *Colletotrichum* (or *Gloeosporium*) species. They are hyaline (pinkish in mass), non-septate and oblong with rounded ends. The size of spores varies from 10—22 x 4—5 μ with an average of 15.9 x 4.7 μ (for 20).

The aerial hyphae also produce spores at their free ends resembling those borne in the acervulus.

GROWTH ON APPLE

A green apple was surface sterilised, inoculated with a two-day old culture and kept in a moist chamber. Two days after inoculation a dark-brown rot was found all around the inoculum. A few days later the discoloured surface was covered with black pustules with groups of black setae in the centre. On maturity the black pustules burst open showing the pink masses of spores, characteristic of the acervuli with encircling setae. Sometimes an acervulus was found unassociated with any setae, or a group of setae without an acervulus.

In ten days the rot was 1.5 inches in diameter. The spores borne on setae measured 19—24 x 4—6 μ with an average of 20.9 x 5.3 μ (for 25 spores).

The spores produced in acervuli measured 16—24 x 4.2—8 μ with an average of 19.6 x 5.8 μ (for 30 spores).

After 22 days the whole apple was discoloured with the rot. The inoculated apple was kept in the moist chamber at room temperature (about 28°C) for three months, and afterwards at a lower temperature (about 5°C) for a further period of three months in an unsuccessful attempt to produce the perfect stage of the fungus.

PATHOGENICITY OF THE FUNGUS (Plate IB and Plates II & III)

Mature and immature leaves of several young rubber seedlings in a nursery were sprayed with a spore suspension in sterile water, and immediately wrapped in brown paper, which was kept in position for 48 hours. Unsprayed control leaves from the same flush were similarly wrapped. Tests were also carried out with mature and immature leaves of young plants growing in pots in the laboratory, the inoculated and control plants being covered with tall bell-jars, some of which were removed after 24 hours and others after 48 hours.

Three days after inoculation a number of minute black spots were found on leaves which at the time of inoculation had just begun to change from bronze to light green. Five days after inoculation many of the young leaves were found heavily infected, some already distorted with the tips curled up. The characteristic setae, bearing spores, were present on the lower surfaces of many of the lesions. The spots were spherical on the leaf lamina and oval or elongated on the midribs and veins. In the case of some leaves the entire midrib or part of it became black due to the coalescence of large numbers of lesions. Black lesions were also found on the leaf stalks and on the tender green parts of the stem. By the seventh day after inoculation some of the severely infected leaves of the nursery plants dropped. The affected leaves in general had a superficial resemblance to those infected with *Helminthosporium heveae* or *Gloeosporium alborubrum*. Two weeks after inoculation some of the lesions were as large as 3 mm. in diameter; the small spots were uniformly dark brown in colour, while the large ones had greyish white centres, and were surrounded by a yellow halo. In the latter, acervuli arranged almost in concentric circles were present, surrounded by a large number of setae. Tufts of setae were also found associated with unopened acervuli.

Infection was generally more severe in the nursery than in the laboratory, as many as a thousand lesions being found on a single leaflet of a nursery plant. The newly unfolded bronze-coloured leaves, and those just changing from the bronze to the light green stage, were the ones most affected. Younger leaves in a folded condition at the time of inoculation had a smaller number of lesions, and no lesions were found on mature green leaves. The same dependence of the intensity of infection

on the age of the leaf at the time of inoculation was found with the laboratory plants, irrespective of the period of incubation under a bell-jar.

To confirm the identity of the pathogen it was re-isolated from artificially infected leaves and the culture found to resemble in every respect the original isolation with which the inoculations were made. In all cases uninoculated leaves remained free from infection. It is thus evident from these experiments that the fungus is parasitic and capable of causing severe infection, which under favourable conditions results in leaf-fall.

Relationship to similar fungi

The fungus here described resembles both *Colletotrichum gossypii* Southworth (1891) and *C. derridis* van Hoof (1950) in bearing spores on setae, and in having similar spore measurements. In Table I a comparison is made of these species and the two species of *Colletotrichum* already recorded on rubber.

C. derridis, which is widespread on derris in Malaya was isolated from *Derris elliptica* (var. Changi 3) for comparison with the *Hevea* fungus. On most media, the two strains resembled each other closely in general appearance, but in every case the former grew more slowly. Moreover, the derris isolate did not produce acervuli on agar media whereas the other did so on Difco potato dextrose, corn meal, and oat agars. Both produced acervuli in abundance on sterilised green rubber twigs.

Inoculation experiments with the derris isolate failed to produce leaf spots on rubber, although it was found to infect *Derris elliptica* (var. Changi 1) producing fairly large lesions. On the other hand inoculation experiments with the *Hevea* fungus resulted in infection of derris, but the lesions were much smaller.

TABLE I *Comparison of species of Colletotrichum similar to R.R.I. culture No. 288*

Name	Described by	Spore size	Seta length	Spores on seta	Appressoria	Perfect stage
Colletotrichum ficus	Koorders, S. H. (1905) (1907) -	Normally 10-15 x 3.5-4 μ larger & smaller may be found -	Usually 30-90 μ rarely up to 160 μ -	No	Yes	*Neozimmermannia elasticae* (Zimm) Koord.
Colletotrichum heveae	Petch, T. (1906) -	18-24 x 7.5-8 μ	up to 90 μ -	No		Not known.
Colletotrichum (Gloeosporium) gossypii	Southworth, E. A. (1891) -	11-20 x 4-9 μ -	100-250 μ -	Yes		*Glomerella gossypii* Edgerton C. W. (1909)
Colletotrichum derridis	van Hoof, H. A. (1950) -	10-21 x 4-6.5 μ	20-110 μ -	Yes		Not known.
Colletotrichum R.R.I. Culture No. 288 -		On setae on leaf 13-20 x 4.5-8 μ; in acervulus on leaf 14-22 x 4-6 μ; in acervulus on P.D. agar 10-22 x 4-5 μ -	84-170 μ -	Yes	Yes	Not known.

In the course of the investigations a leaf-spotting *Colletotrichum* very similar to that on rubber was found on *Manihot glaziovii*. This fungus was isolated and compared with the *Hevea* fungus on various agar media as well as on green rubber twigs, and both were similar in all respects. The setae and spore measurements of both were also similar. Cross inoculations of the isolates from *Manihot* and from *Hevea* showed that they were pathologically indistinguishable. From these observations it is concluded that the two fungi are identical.

Although this is the first account of a *Colletotrichum* with sporogenous setae parasitic on *Hevea*, it is considered undesirable to describe it as a new species because of its close morphological resemblance both to Southworth's *C. gossypii* and to van Hoof's *C. derridis*. Because the *Hevea* pathogen is also found to be slightly pathogenic to derris, it is being provisionally regarded as a strain of *C. derridis*.

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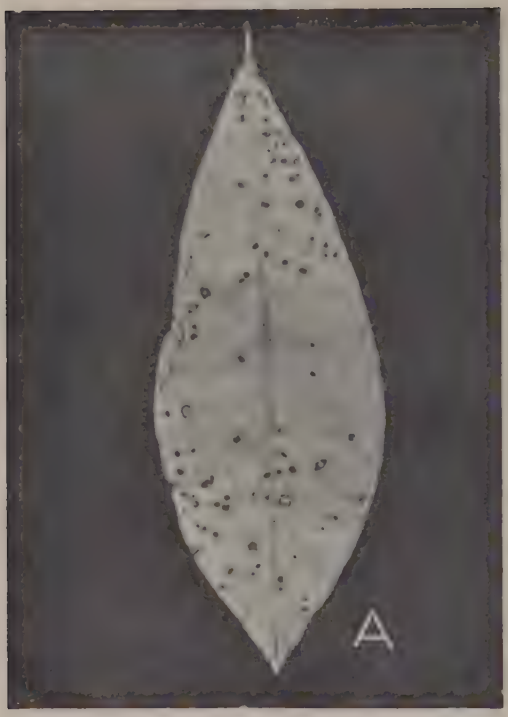
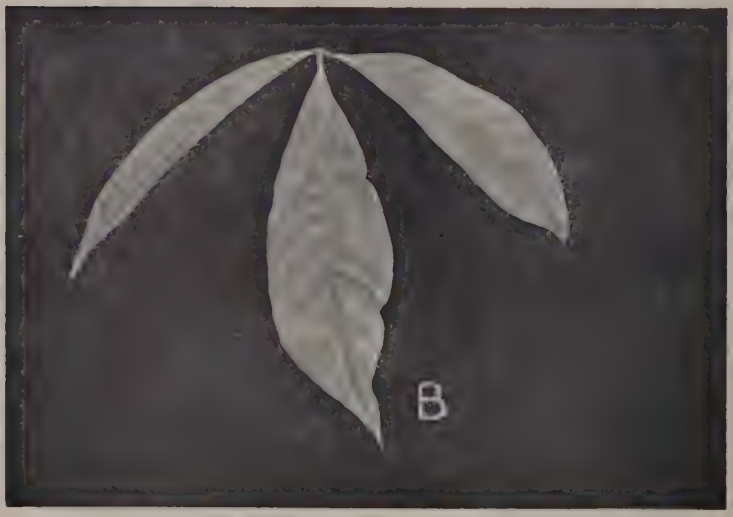
PLATE I



A. Naturally infected leaf with large lesions.



B. Artificially infected leaf of a laboratory plant with small lesions.

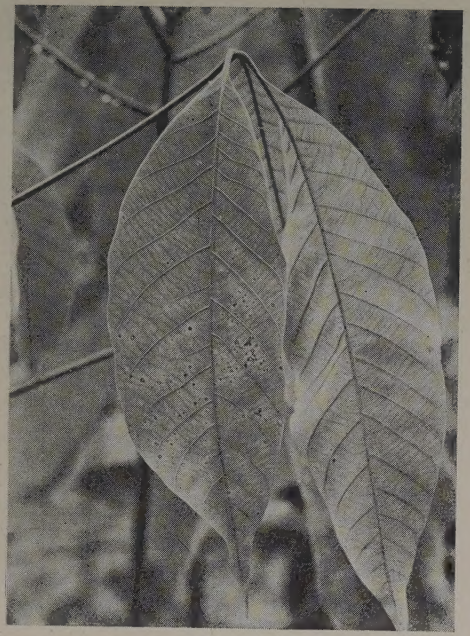


PLATE II

Artificially infected leaf of a nursery plant one week after inoculation.

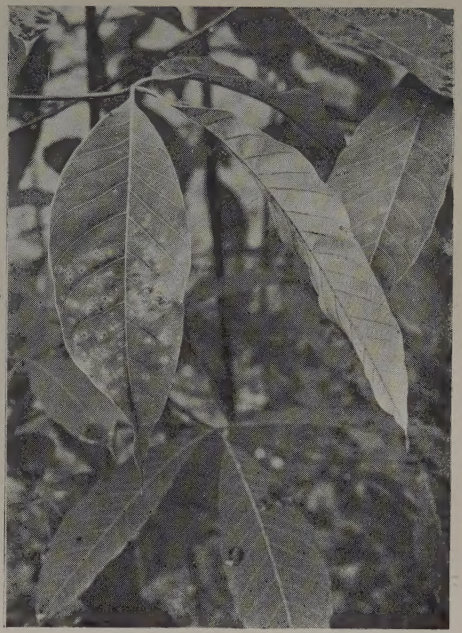


PLATE III

The same leaf 5 weeks after inoculation. Note that the centres of the lesions are turning white with a yellow halo. The central leaflet shows the black lesions on the midrib and veins.

PLATE IV A

Petri dish culture of the fungus on Difco Potato Dextrose agar after 5 days' growth.

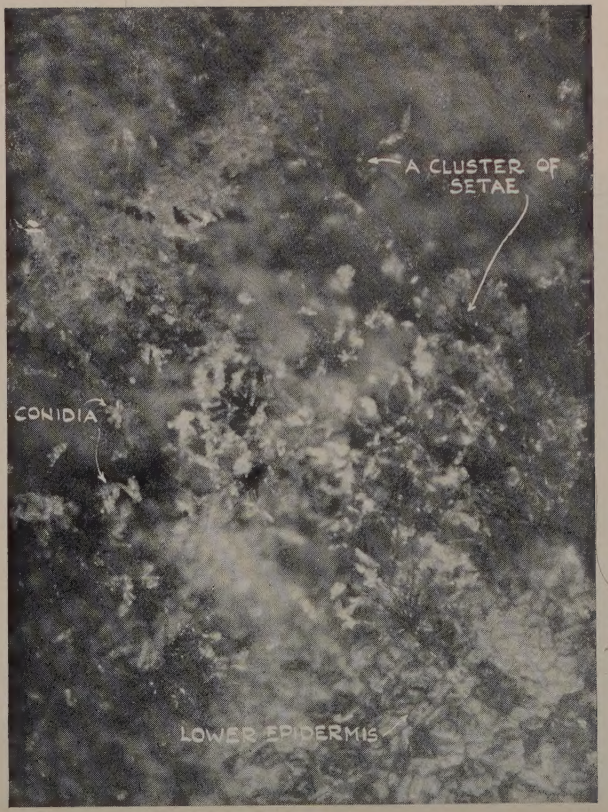


PLATE IV B

Part of a lesion magnified to show the setae with spores.